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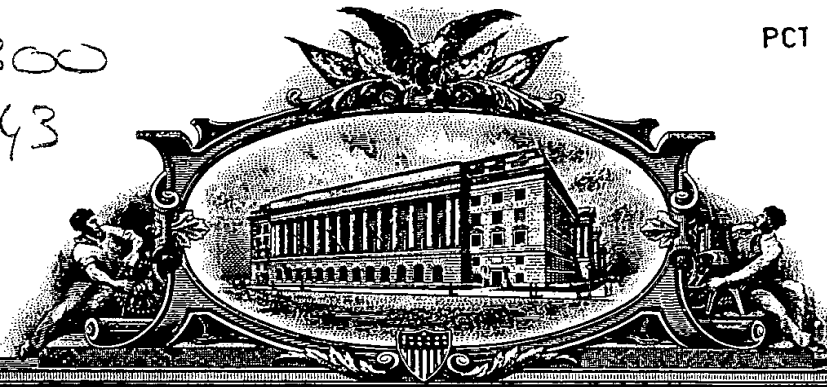
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PROVISIONAL PATENT APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION for patent under 37 CFR 1.53(b)(2).

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TITLE OF THE INVENTION (280 characters max)

PURIFICATION METHOD

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ENCLOSED APPLICATION PARTS (check all that apply)

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| <input checked="" type="checkbox"/> Specification | Number of Pages | <u>10</u> | <input type="checkbox"/> Small Entity Statement |
| <input type="checkbox"/> Drawing(s) | Number of Sheets | <u> </u> | <input type="checkbox"/> Other (specify) |

METHOD OF PAYMENT (check one)

- ☒ A check or money order is enclosed to cover the Provisional filing fees 19400
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- ☒ No
- ☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted

SIGNATURE

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REGISTRATION NO. 22,980

- ☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

Purification method

The present invention relates to an improved method for purification of commercially important sterols from neutral substances or refined neutral substances, which have been separated from soaps.

Sterols useable in pharmaceuticals and foods need to be purified to quite a high degree to exclude undesired components, such as inorganic salts and soap residues existing in the neutral substances. Although usually present in low concentrations, a part of these impurities usually tend to remain in the sterol fraction, if the isolation of sterols has been performed by known straight crystallization processes without any particular purification steps. The neutral substances in most cases contain also organic impurities, which usually make direct crystallization of sterols in pure form difficult. One kind of impurities is the group of compounds, which typically cannot be seen in gas chromatographic analyses usually used in sterol assay. The exact nature of these compounds is not known, but there is some evidence that this group consists of a wide molecular weight range hydrocarbon type material. Because of the invisibility in gas chromatographic analysis, the material is often called "non-elutable compounds" or "nonelutables". Also the lighter components of the neutral substances may sometimes interfere crystallization of sterols. In a typical case, when the neutral substances are originated from wood pulping, this light fraction typically consists of, for instance, di- and sesquiterpene compounds, stilbenes and wax alcohols.

The separated sterol product obtained by the method according to the invention contains practically only sterol components present in the neutral substance, without any interfering salt or organic impurities, which would make crystallization purification difficult. A further advantage of the process according to this invention is that in addition to the high purity simultaneously very high sterol yields are obtained.

The neutral substances (unsaponifiables or neutrals) can be obtained by extracting from soaps. Any soap of vegetable origin may be used. Neutral substances suitable as raw material in the method according to the invention may therefore be obtained from extracts of vegetable origin such as soaps from vegetable oils, or preferably from crude soap from

the sulphate cellulose process or pitch soap obtained from tall oil. The method according to the present invention is preferably suitable for neutral substances obtained from soap originated from wood pulping.

The neutral substances can be obtained from soaps typically by extraction. Extraction of these neutral substances from soaps can be performed, for example, using hydrocarbon solvents at elevated temperatures and pressures, or using mixtures of hydrocarbons and ketones and/or lower alcohols as extraction solvents (e.g. US 3 965 085).

Prior sterols have been crystallized from sterol containing materials, typically from neutral substances, using alcohol, ketone or hydrocarbon solvents without or with water (US 2704 764, US 2729655, US 2843610, US 2870176, US 5117016, US 4 420 427). None of these methods work quite satisfactorily in the most usual cases where the substance that contains sterols also contains said impurities.

This invention relates to a method for producing in good yields high quality, high purity sterols, the method comprising extraction of neutral substances from soaps, removal of components interfering sterol separation from the said neutrals, and crystallization from the purified neutral substance.

As previously mentioned, the extraction of neutral substance from soaps can take place using a hydrocarbon solvent as extraction solvent. If sole hydrocarbon is used as solvent, the extraction may preferably be performed at elevated temperatures (and pressures) in order to break the emulsions otherwise formed.

Typical extraction conditions are, for example:

- the hydrocarbon used as extraction solvent is heptane,
- the solids content in the soap to be extracted is about 25-40%,
- the amount of the solvent used in the extraction is more than one part per one part of dry soap by weight,
- the extraction temperature is higher than 140 °C (whereby the pressure will be typically more than 10 bars)

- the extraction can be performed using any known extraction method (batch extraction, column, mixer-settler etc.)

It is well known from the prior art that the emulsions can be avoided also by using ketones or lower alcohols together with hydrocarbons as the extraction solvents, for example according to US patent 3 965 085. In this case high temperatures and pressures can be avoided but, on the other hand, in industrial applications the solvent recoveries will become more complicated.

It has now been realized that in order to remove the impurities interfering sterol crystallization from the extracted neutral substance, it may be necessary to apply various processes/process combinations depending on the type of impurity to be removed. The unit operations in question connected with this invention are

- a) high temperature water wash of neutral substance, and
- b) evaporation fractionation of the impurities in the neutral substance.

The method according to the invention is more precisely characterized by the elements disclosed in the claims.

By high temperature water wash the traces of inorganic salts and soap residues can be removed. These impurities typically exist only in low concentrations, but give the sterol product, if crystallized without removing them, an ugly gray or brownish color. The soaps present in the neutrals usually make also filtration of the crystallized sterols difficult. The high temperature water wash of neutrals has preferably to be performed at high temperature and high pressure if there is any tendency of emulsion formation during the wash. The process can be performed as an extraction of neutral substance dissolved in hydrocarbon solvent with water by using any suitable known extraction technique. Preferably, the water wash may be done directly after a high temperature hydrocarbon extraction of soap (if this technique is applied for producing neutral substance) and applying approximately the same conditions, i.e. temperature between about 120 °C and 190 °C and the pressure prevailing at that temperature. The amounts of water to neutral substance (hydrocarbon) phase may vary in a broad range; e.g. ratios from 1:10 to 10:1

Evaporation fractionation of impurities can be applied connected to the water wash, or it can be used instead of it. By using short path distillation (e.g. thin film or wiped film) the components lighter than sterols can first be removed. The typical applicable conditions are for example 0.1 mbar pressure and 160 °C temperature. The evaporation removes for example diterpene compounds, stilbenes and wax alcohols interfering the subsequent crystallization of sterols.

The removal of these components can further be improved by using a rectification column in connection with the short path evaporation.

The content of these impurities in typical neutral substances originated e.g. from wood pulping, may be up to 30-40%. The sterols can be crystallized from the evaporation bottoms. The advantage is that because of increased sterol content in the thus refined neutral substance and removal of interfering components the yield and the purity of the sterol product will be better than without evaporation fractionation. However, this type of evaporation does not remove inorganic salts and soap residues. Thus, to get still better result it is advantageous to combine this evaporation with high pressure water wash pretreatment.

The evaporation fractionation of the neutral substance can also be implemented so that the sterol-rich fraction is evaporated and accordingly separated from the heaviest components of the neutral substance. In this case also inorganic salts and soap residues will be separated from the sterol fraction. No separate water wash procedure is needed in this case. Evaporation of the light fraction can be a preceding part of the evaporation of the sterol fraction in order to most efficiently fractionate the interfering impurity components. Typical conditions for evaporation of the sterol rich fraction are e.g. pressure 0.1 mbar, temperature 220 °C. It has been shown that the sterols contained in the evaporation feed can be recovered practically quantitatively to the distillate when having

proper distillation conditions. Thus, no significant sterol losses will take place during this purification step. Taking into account that the sterol content in the sterol rich fraction during evaporation fractionation can be increased typically from ~ 10% content in the original neutral substance to over 40-60% in the fractionated sterol rich distillate fraction, and this taking place without any substantial sterol losses, it is obvious that this purification offers a very good starting point for the crystallization purification step of sterols.

Crystallization of the sterols from the sterol rich fraction produced with said means can be performed by using any known solvent or solvent combination. However, particularly advantageous is to use aliphatic or cycloaliphatic hydrocarbons combined with water, or even more preferably may be the use of combinations of said hydrocarbons, water and lower alcohol, especially methanol.

It has now been shown that sterols can be separated from neutral fractions by crystallization from the mixtures of hydrocarbon and water or from mixtures containing hydrocarbon, water and lower alcohol, especially methanol. When the crystallization takes place directly after extraction it is not necessary to evaporate the hydrocarbon (or hydrocarbon mixture) totally to dryness before the crystallization process, but it is possible to add water and optionally lower alcohol directly to the concentrated hydrocarbon phase. However, hydrocarbon must be evaporated, if further purification of neutral substance prior to crystallization is needed.

It has also been noticed that water plays a significant role in the crystallization of sterols from said neutrals. If the solvents have been evaporated totally from the hydrocarbon phases obtained from the extraction and thereby also water has been removed, the crystallization from this dry neutral fraction using hydrocarbon or hydrocarbon/alcohol mixtures without water as the solvents gives usually only very small yields of sterols. However, when the neutrals are in the extraction solvent or in partly evaporated extraction solvent, the yields of the crystallized sterols are usually reasonable good. In this case the hydrocarbon phase probably contains some water originated from the soap

phase of the extraction, and this water helps improving the yield in next crystallization step.

The role of the optionally added alcohol in the crystallization mixture is to improve the crystal shape so that the separation of crystals by e.g. filtration in many cases becomes much easier. Usually, if the neutral substances have been purified by any further steps, alcohol addition improves also the purity of the separated sterol product.

If the neutral substances are not completely dried from the hydrocarbon solvent they have been solved into during the extraction process, less of another solvent needs to be used in the separation process. Therefore, the extraction solvent containing the extracted neutral substances may according to the present invention preferably be used as such or partly evaporated. A suitable ratio of neutral substances in dry weight to the amount of extraction solvent, or solvent mixture is 1:1-12 by weight.

Preferable hydrocarbons in the invention include hydrocarbons containing 1-10 carbon atoms such as straight chain hydrocarbons (preferably hexane, heptane or octane), branched chain hydrocarbons, hydrocarbons containing cycloaliphatic structures or mixtures comprising any of these.

The preferable amount of optionally added lower alcohol, e.g. methanol or ethanol or their mixtures, most preferably methanol, is about 1-20% by weight of the amount of neutral substances (dry weight).

The crystallization process can be performed as follows:

The mixture of solvents and neutral substance is heated (if it is not warm enough as it may be in the case the stream is brought directly from extraction, solvent concentration, or solvent adjusting steps) to about 60-80°C and cooled after that to 0-40°C, preferably to 20-30°C to crystallise the desired sterols. The crystals are collected by e.g. filtration or centrifugation.

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The obtained sterol crystals may be purified by any known methods including e.g. washing and/or recrystallization, and if both is needed, they may be performed in optional order.

Preferably the collected crystals may be washed. By washing with a hydrocarbon, preferably a solvent similar to the one used for the extraction e.g. hexane or heptane, nonpolar impurities are removed. Preferably the washing solvent may be a solvent mixture of the same composition as the the one used as crystallisation solvent, or – if it will separate into two separate layers, the hydrocarbon phase of that solvent mixture.

Preferably also a washing step with water may be performed. In this the crystals are solved in hydrocarbon, preferably again e.g. heptane, and heated to about 60-80oC. Water is added at 60-80oC in an amount of 0,1-2 times the total mass. The water phase containing impurities is then removed.

Alternatively or in addition, if needed, a recrystallisation step may be performed to purify the sterols further. In the recrystallisation, although the same solvent compositions can be used as in the first crystallisation, more advantageous is to use solvent compositions, which are richer in lower alcohol (especially methanol) and contain only 5-20% hydrocarbon solvent and optionally a small amount of water (0-5%). This methanol rich solvent in recrystallisation will give crystals which are easier to separate by e.g. filtration than if the crystallisation was performed from a hydrocarbon rich solvent system.

The following examples explain the invention in more detail. The %-figures mean weight-%. The term purity means the content of identified sterol components. The sterol product contains also small amounts of probably closely related, but not exactly identified sterol like components, which behave like the sterols in question and cannot be removed using these processes. Thus, the maximum purity practically attainable by these purification processes is about 98%.

Example 1.

The unsaponifiables (from *Pinus taeda* based pitch soap) used in this crystallization were in a hydrocarbon solvent (a mixture of aliphatic and cycloaliphatic) hydrocarbons, LIAV110 delivered by Neste Oy). The mixture of solvent and unsaponifiables was brought directly from the soap extraction, which was performed at 170 °C and 18 bar pressure in a pressure autoclave. The material ratios of the components were 1 part dry soap, 2 parts water and 4 parts solvent. After 5 minutes at extraction temperature mixing was stopped and the layers were allowed to separate. The lower water phase was separated through a cooled sampling bomb when the contents was still hot. Solids content in the remaining hydrocarbon phase was 11,3% and the sterols content 35% of the solids. The hydrocarbon phase was allowed to cool slowly to 20 °C. The precipitated sterols were filtered and washed with fresh solvent. From 100 g of dry neutrals was obtained 22,5 g of a sterol blend consisting of sitosterol, sitostanol, campesterol and campestanol and nonelutable impurities. The color of the product was light greybrown. The ash content describing the amount of inorganic salts was 0.4%. The sterol content was 80%.

Example 2. The effect of water wash on the purity and ash content of sterols.

10 l of the hydrocarbon phase of example 1 was put into an autoclave and 5 l water was added. The autoclave was closed and the temperature was raised to 130°C simultaneously stirring the contents. After 5 minutes at 130 °C the stirring was stopped and the lower water phase was separated through a water cooled sampling bomb. The hydrocarbon phase was let to cool to 20 °C. The crystallized material was filtered and washed with fresh hydrocarbon. The color of the resulting crystalline crude sterol was white and the ash content was 0.09%. The purity of the sterols was 82% and the yield 23 g/100 g feed neutral substance.

Example 4. This example shows the effect of evaporation fractionation on the crystallisation result.

The *Pinus radiata* unsaponifiables extracted using a commercial hexane-acetone-extraction (solvent content 5%) were dried from residual solvents using short path evaporation (the equipment was the wiped film evaporator KDL-5 manufactured by UIC). The conditions were: pressure 10 mbar and evaporator temperature 100 °C in the jacket of

crystallized and separated from a LIAV/methanol/water solvent as in example 4. The sterol yield was 80% and purity 97.5%. Ash content of the almost white product was 0.1%.

Example 7. This example describes the possibilities to maximize sterol recoveries in the evaporation fractionation processes.

The feed material was *Pinus radiata* wood based unsaponifiables extracted by hexane-acetone solvent extraction. The dried unsaponifiables were fed to a wiped film evaporator equipped with a rectification column. The temperature of the feed neutrals was 150 °C, the temperature in the bottom part of the column 207 °C, in the top part of the column 189 °C, and in the residue 241 °C. (The jacket temperature was 298 °C). The pressure inside the equipment was 2.2 mmHg. The amount of light fraction distilled over was according to the measurements 23% and the amount of residue 76%. The sterol loss into the light fraction was only 0.4%. Sterol content in the residue was 48.1 %.

The residue from this distillation was fed into a short path distillation equipment. The conditions there were: pressure 0.1 mmHg, feed temperature 149 °C, jacket temperature 312 °C, condenser temperature 112 °C, temperature of residue 184°C. The sterol yield of the distillate in this evaporation was 95% of the feed mass (residue 5% of the feed). The sterol concentration in the distillate was 51.5% and in the residue 9%. The loss of sterols in this distillation was only 1%. A sample of the distillate was dissolved in a LIAV110/methanol/water solvent and sterols were crystallised, separated and washed with a solvent similar to the one used in the crystallization as in previous examples. The sterol yield was 83% and purity 97.8%. Ash content of the product was 0.01%.

Claims:

1. A method for isolating sterols from soaps comprising
 - extracting the neutral substances from soap
 - optionally washing the neutral substances with water
 - performing an evaporation fractionation to separate a sterol rich fraction
 - crystallizing the sterols from a suitable solvent or solvent system
 - isolating the sterols.

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